

a.) Amendment to the Claims

1. (Currently Amended) A method for inducing differentiation of an embryonic stem cell into ~~an ectodermal cell~~ a neural stem cell or a nerve cell *in vitro*, which comprises culturing the embryonic stem cell under non-aggregation conditions, wherein said culturing is carried out in the absence of retinoic acid, and in the presence of a stroma cell ~~or a stroma cell-derived factor~~.

Claims 2-13 (Cancelled).

14. (Currently Amended) The method according to ~~any one of claims 1, 12, 13 and 73~~ claim 1, wherein the non-aggregation conditions are conditions not mediating an embryoid body.

15. (Currently Amended) The method according to ~~any one of claims 1, 12, 13 and 73~~ claim 1, which further comprises culturing under serum-free culture conditions.

Claims 16-17 (Cancelled)

18. (Currently Amended) The method according to ~~any one of claims 1, 12, 13 and 73~~ claim 1, wherein the stroma cell is a stroma cell whose proliferation potency is deleted by a physicochemical treatment.

19. (Currently Amended) The method according to ~~any one of claims 1, 12, 13 and 73~~ claim 1, wherein the ~~physicochemical treatment is selected from the group consisting of an antitumor agent irradiation and~~ stroma cell is a stroma cell whose proliferative potency is deleted by an antitumor agent, irradiation or pathologic tissue fixative.

20. (Currently Amended) The method according to ~~any one of claims 1, 12, 13 and 73~~ claim 18, wherein the physiocochemical treatment is an antitumor agent selected from the group consisting of mitomycin C, 5-fluorouracil, adriamycin and methotrexate.

21. (Currently Amended) The method according to ~~any one of claims 1, 12, 13 and 73~~ claim 1, wherein the ~~physicochemical treatment is selected from the group consisting of~~ stroma cell is a stroma cell whose proliferative potency is deleted by a microwave fixation, a rapid freeze-substitution fixation, a glutaraldehyde fixation, a p-formaldehyde fixation, a formalin fixation, an acetone fixation, a Van fixation, a periodic acid fixation, a methanol fixation and an osmic acid fixation.

22. (Currently Amended) The method according to ~~claim 1~~ claim 23, wherein the stroma cell is recognized by a monoclonal antibody produced by hybridoma FERM BP-7573.

23. (Currently Amended) The method according to any one of claims 1, ~~12, 13 and 73~~ 14, 15 and 18-21, wherein the stroma cell is selected from the group consisting of: a fetal primary culture fibroblast; an SIHM mouse-derived STO cell; a mouse fetus-derived NIH/3T3 cell; an M-CSF deficient mouse calvaria-derived OP9 cell; a mouse calvaria-derived MC3T3-G2/PA6 cell; an embryonic stem cell-derived stroma cell; and a bone marrow mesenchymal stem cell-derived stroma cell.

24. (Currently Amended) The method according to ~~any one of claims 1, 12, 13 and 73~~ claim 1, wherein the embryonic stem cell is selected from the group consisting of:

(a) an embryonic stem cell established by culturing an early embryo before implantation;

(b) an embryonic stem cell established by culturing an early embryo produced by nuclear transplantation of the nucleus of a somatic cell; and

(c) an embryonic stem cell in which a gene on the chromosome of the embryonic stem cell of (a) or (b) is modified using gene engineering.

25. (Canceled)

26. (Currently Amended) The method according to ~~any one of claims 1, 12, 13 and 73~~ claim 1, wherein the embryonic stem cell differentiates into an ectodermal cell or an ectoderm-derived cell at an efficiency of 5% or more.

27. (Currently Amended) The method according to any one of ~~claims 1, 12, 13 and 73~~ claim 1, which does not substantially accompany differentiation induction of a mesodermal system cell.

Claims 28-55 (Cancelled)

56. (Currently Amended) A method for evaluating a substance for activity in regulating differentiation of an embryonic stem cell into an ectodermal cell or an ectoderm-derived cell, which comprises:

carrying out the method according to ~~any one of claims 1, 2, 12, 13~~
~~or 22~~ either of claims 1 or 24 both (i) in the presence of a substance to be tested and (ii) in
the absence of the substance to be tested; and

comparing differentiation of the embryonic stem cell into an
ectodermal cell obtained in the presence of the substance to be tested with that in the
absence of the substance to be tested.

57. (Currently Amended) A method for screening a substance for activity
in regulating differentiation of an embryonic stem cell into an ectodermal cell, which
comprises:

carrying out the method according to ~~any one of claims 1, 2, 12, 13~~
~~or 22~~ either of claims 1 or 24 both (i) in the presence of a substance to be tested and (ii) in
the absence of the substance to be tested; and

comparing differentiation of the embryonic stem cell into an
ectodermal cell obtained in the presence of a substance to be tested with that in the absence
of the substance to be tested.

Claims 58-71 (Cancelled).

72. (Currently Amended) The method according to ~~any one of claims 1,~~
~~12, 13 and 73~~ either of claims 1 or 24, wherein the stroma cell is PA6, OP9 or NIH3T3.

Claim 73 (Cancelled)

74. (New) The method according to claim 23, wherein the embryonic stem cell is selected from the group consisting of:

- (a) an embryonic stem cell established by culturing an early embryo before implantation;
- (b) an embryonic stem cell established by culturing an early embryo produced by nuclear transplantation of the nucleus of a somatic cell; and
- (c) an embryonic stem cell in which a gene on the chromosome of the embryonic stem cell of (a) or (b) is modified using gene engineering.

75. (New) The method according to claim 74, wherein the stroma cell is recognized by a monoclonal antibody produced by hybridoma FERM BP-7573.

76. (New) The method according to claim 56, wherein the stroma cell is selected from the group consisting of: a fetal primary culture fibroblast; an SIHM mouse-derived STO cell; a mouse fetus-derived NIH/3T3 cell; an M-CSF deficient mouse calvaria-derived OP9 cell; a mouse calvaria-derived MC3T3-G2/PA6 cell; an embryonic

stem cell-derived stroma cell; and a bone marrow mesenchymal stem cell-derived stroma cell.

77. (New) The method according to claim 76, wherein the embryonic stem cell is selected from the group consisting of:

(a) an embryonic stem cell established by culturing an early embryo before implantation;

(b) an embryonic stem cell established by culturing an early embryo produced by nuclear transplantation of the nucleus of a somatic cell; and

(c) an embryonic stem cell in which a gene on the chromosome of the embryonic stem cell of (a) or (b) is modified using gene engineering.

78. (New) The method according to claim 57, wherein the stroma cell is selected from the group consisting of: a fetal primary culture fibroblast; an SIHM mouse-derived STO cell; a mouse fetus-derived NIH/3T3 cell; an M-CSF deficient mouse calvaria-derived OP9 cell; a mouse calvaria-derived MC3T3-G2/PA6 cell; an embryonic stem cell-derived stroma cell; and a bone marrow mesenchymal stem cell-derived stroma cell.

79. (New) The method according to claim 78, wherein the embryonic stem cell is selected from the group consisting of:

(a) an embryonic stem cell established by culturing an early embryo before implantation;

(b) an embryonic stem cell established by culturing an early embryo produced by nuclear transplantation of the nucleus of a somatic cell; and

(c) an embryonic stem cell in which a gene on the chromosome of the embryonic stem cell of (a) or (b) is modified using gene engineering.